Trace levels of 22 ß-lactam antibiotics, including penicillins, cephalosporins and carbapenems in Animal Tissue and Fluids by LC-MS/MS

Instrumentation: Waters Acquity UPLC system **with** AB Sciex LC/MS/MS System
Column: Waters Acquity UPLC CSH C18 analytical column of 2.1 × 100 mm, 1.7 µm
Elution Type: Gradient
Mobile Phase A: 0.0032 % ammonia in water
Mobile Phase B: 0.0032 % ammonia in water/acetonitrile (1:9 v/v)
Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	100	0
2	1	100	0
3	9.0	60	40 (Curve 6)
4	10.0	0	100 (Curve 6)
4	10.5	0	100

Flow Rate: 0.4 mL/min
Col. Temp: 50 °C
Inj. Vol.: 10 µL
Detection: Tandem Mass Spec (MS-MS) LC/MSD Trap (Ctrl + Click to follow link)
Detector Info: Applied Biosystem 3200 Q TRAP LC/MS System (Ctrl + Click to follow link)
MS Conditions
Source: Positive ESI
Source temp.: 150 °C
Nebulizer: 40 psig
Desolvation Gas flow: 10 L/min
Desolvation Gas temp: 550 °C
Vcap: 2000 V
SRM (MRM): Instrument Acquisition Data Used for the Analysis of Antibiotics:

Analyte	Precursor ion (m/z)	Product ion (m/z)	DP	Collision energy (eV)
Penicillin G	420.2	261.2	40	15
		176.0	40	20
Penicillin G-d_7	427.2	160.0	40	20
Ampicillin	435.2	286.0	40	20
		276.2	40	20
Ampicillin-d_5	440.2	291.0	40	20
Penicillin V	436.0	277.0	40	20
		192.0	40	20
		160.0	40	20
Penicillin-V-d_5	441.0	160.0	40	20
Amoxicillin	451.2	275.2	40	15
		302.2	40	15

SRM (MRM): Instrument Acquisition Data Used for the Analysis of Antibiotics (cont.):

Analyte	Precursor ion (m/z)	Product ion (m/z)	DP	Collision energy (eV)
Amoxicillin-d_4	455.2	279.2	40	15
Oxacillin	487.0	160.0	40	17
		328.2	40	15
		243.1	40	20
Nafcillin	500.0	256.2	40	15
		199.1	40	22
Cloxacillin	521.2	160.0	40	20
		362.2	40	15
Dicloxacillin	555.0	160.0	40	20
		396.0	40	15
Dicloxacillin-$^{13}C_4$	559.0	160.0	40	20
Cephalexin	276.2	143.1	40	22
		259.1	40	22
		231.2	40	22
Cephalexin-d_5	281.2	264.1	40	22
Cephapirine	294.1	152.0	40	24
		181.0	40	22
		143.1	40	22
Cephapirine-d_4	298.1	156.0	40	24
Ceftiofur /	326.1	169.0	40	19
Cefquinome		241.0	40	17
		213.0	40	22
Ceftiofur-d_3	329.1	169.0	40	17
Cefacetrile	468.2	339.2	20	19
		424.2	20	17
Cefazolin	493.2	294.1	40	22
		336.2	40	22
Cefazolin-$^{13}C_2$-^{15}N	496.2	296.1	40	22
Cefalonium	507.2	378.2	45	24
		463.4	45	19
Ceforperazone	718.3	386.3	40	27
		630.4	40	22
Imipenem	358.1	314.1	35	17
		172.1	35	24
		154.2	35	37
Imipenem-d_4	362.1	318.1	35	17
Faropenem	371.1	154.2	55	21
		172.1	55	17
		257.1	55	23
Biapenem	399.2	154.1	50	35
		172.1	50	31

SRM (MRM): Instrument Acquisition Data Used for the Analysis of Antibiotics (cont.):

Analyte	Precursor ion (m/z)	Product ion (m/z)	DP	Collision energy (eV)
Meropenem	469.2	207.1	20	27
		425.2	20	13
Meropenem-d_6	475.2	207.1	20	27
Doripenem	506.0	207.1	30	29
		462.2	30	19
Ertapenem	560.9	517.1	40	23
		207.2	40	31
Ertapenem-d_4	564.9	207.2	40	31

Sample Preparation:

Samples were extracted, shaken and centrifuged. The aqueous layer was transferred into a clean test tube and neutralised (pH 7.2) by adding acetic acid (25 %) and/or ammonia (2.5 %) and the sample was centrifuged again (3500 g, 15 min). A Phenomenex Strata-X 200 mg / 6 mL reversed phase solid phase extraction (SPE) cartridge was conditioned with 5 mL MeOH and 5 mL water. The clear neutralised extract was applied onto the SPE cartridge which was subsequently washed with 5 mL of MeOH/water (1:9 v/v) and dried by applying vacuum for 5 min. The ß-lactam hydrolysis products were eluted from the cartridge using 5 mL MeOH/ACN (50:50, v/v) followed by evaporation of the solvent (45 °C, N2). The residue was redissolved in 500 µL 1 % piperidine in water
and transferred into an LC-MS/MS sample vial.

Results:

The calculated decision limits (CCα) based on zero tolerance, indicating the limit of detection, vary from 0.7 – 20 µg/kg. The penicillins have detection limits in the low ppb range demonstrating that the method is suitable for the detection of penicillins at levels well below the MRL enabling the monitoring of extra-label penicillin use. The method is also suitable for the detection of cephalosporins and carbapenems at relevant levels. The calculated detection capabilities (CCß) are between 1.5 and 50 µg/kg.

References:

B.J.A. Berendsen, H.W. Gerritsen, R.S. Wegh, R. van Sebille, S. Lameris, A.A.M. Stolker, M.W.F. Nielen, Comprehensive analysis of ß-lactam antibiotics including penicillins, cephalosporins and carbapenems in poultry muscle using liquid chromatography coupled to tandem mass spectrometry, Anal. Bioanal. Chem. (2013) In press, DOI: 10.1007/s00216-013-6804-6.

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Bjorn J.A. Berendsen, PhD thesis, Wageningen University, Wageningen, NL (2013)